

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for evaluating the risk of cancer in a smoker by determining the concentration of circulating total DNA in a plasma specimen from said smoker, which comprises:

- 1) extracting the DNA from the plasma specimen obtained from a patient subject who smokes to create a target DNA sample;
- 2) adding to the target DNA sample: a) a mixture of oligonucleotide primers suitable for PCR amplification of a fragment of the human telomerase reverse transcriptase (hTERT) gene, wherein said fragment of the hTERT gene is from nucleotide position 13059 to nucleotide position 13156 of the sequence of GenBank accession no. AF128893, and b) an oligonucleotide probe, having at least one quencher and one reporter fluorophore at the 3' and 5' ends, able to anneal to a sequence within the region delimited by the primers, in suitable conditions for carrying out a PCR reaction,
- 3) adding a heat-stable DNA polymerase with 5'-3' exonuclease exonuclease activity and amplifying the hTERT gene fragment;
- 4) measuring the produced fluorescence;
- 5) quantifying the hTERT DNA copy number in the target DNA sample interpolating a calibration curve created with known amounts of DNA, wherein the concentration of circulating total DNA in a plasma sample is determined by quantification of hTERT copy number
- 6) correlating the hTERT copy number to the risk of cancer in the patient subject.

2. (Cancelled).

3. (Previously Presented) A method as claimed in claim 1, which further comprises comparing the concentration of circulating DNA to a reference concentration.

4. (Previously Presented) A method according to claim 3, wherein the reference concentration is from 9 to 25 ng/ml.

5. (Cancelled).

6. (Previously Presented) A method according to claim 1, wherein said fragment of the human telomerase reverse transcriptase (hTERT) gene is amplified using SEQ ID NO: 1 and 2 as the primers forward and reverse, respectively, and SEQ ID NO: 3 as the probe.

7. (Currently amended) [[A]] The method as claimed in claim 12, wherein the subject is a cancer patient and said method is further used for the early diagnosis, prognosis or clinical monitoring of said cancer patient cancer patients.

8-9. (Cancelled)

10. (Original) A method as claimed in claim 1, wherein said cancer is lung, colon-rectum, head and neck, liver or pancreas cancer.

11. (Original) A method as claimed in claim 10, wherein said cancer is lung carcinoma.

12. (Currently amended) A method of evaluating the risk of developing cancer in a patient subject by determining the concentration of circulating total DNA in a plasma specimen from the patient subject, which comprises:

- 1) extracting the DNA from the plasma specimen obtained from the patient subject to create a target DNA sample;
- 2) adding to the target DNA sample: a) a mixture of oligonucleotide primers suitable for PCR amplification of a fragment of the human telomerase reverse transcriptase (hTERT) gene, wherein said fragment of the hTERT gene is from nucleotide position 13059 to nucleotide position 13156 of the sequence of GenBank accession no. AF128893, and b)

an oligonucleotide probe, having at least one quencher and one reporter fluorophore at the 3' and 5' ends, able to anneal to a sequence within the region delimited by the primers, in suitable conditions for carrying out a PCR reaction,

- 3) adding a heat-stable DNA polymerase with 5'-3'~~hexonuclease~~ exonuclease activity and amplifying the hTERT gene fragment;
 - 4) measuring the produced fluorescence;
 - 5) quantifying the hTERT DNA copy number in the target DNA sample interpolating a calibration curve created with known amounts of DNA, wherein the concentration of circulating total DNA in a plasma sample is determined by quantification of hTERT copy number
 - 6) correlating the hTERT copy number to the risk of cancer in patient the subject.
13. (Currently amended) The method of claim 12, wherein the patient subject is a healthy individual.
14. (Currently amended) The method of claim 12, wherein the patient subject is an individual with familial cancer susceptibility.